Lipophilic Nalmefene Prodrugs to Achieve a One-Month Sustained Release

Tim Gaekens[a], Michel Guillaume[a], Herman Borghys [b], Loeckie L. De Zwart [c], Ronald de Vries [c], Roger C.A. Embrechts [d], An Vermeulen [e], Anton A.H.P. Megens [b], Josée E. Leysen [b,f], Piet Herdewijn [g], Pieter P. Annaert [h] and John R. Atack [b,*]

[a] API Small Molecule Development, Janssen Pharmaceuticals, Turnhoutseweg 30, B-2340 Beerse, Belgium

[b] Neuroscience, Janssen Pharmaceuticals, Turnhoutseweg 30, B-2340 Beerse, Belgium

[c] Drug Metabolism and Pharmacokinetics, Janssen Pharmaceuticals, Turnhoutseweg 30, B-2340 Beerse, Belgium

[d] Drug Product Development, Janssen Pharmaceuticals, Turnhoutseweg 30, B-2340 Beerse, Belgium

[e] Clinical Pharmacology, Janssen Pharmaceuticals, Turnhoutseweg 30, B-2340 Beerse, Belgium

[f] Department of Nuclear Medicine and PET Research, VU University Medical Centre, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands

[g] Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, Box 1030, B-3000 Leuven, Belgium

[h] Laboratory for Pharmacotechnology and Biopharmacy, Katholieke Universiteit Leuven, O&N2, Herestraat 49-Box 921, B-3000 Leuven, Belgium

* Address correspondence, proofs and reprint requests to Prof. John R. Atack at his current address:
Sussex Drug Discovery Centre
School of Life Sciences
Room 2R314B, Chichester II
University of Sussex
Brighton, BN1 9QJ
United Kingdom
Tel: +44-(0)1273-872722
E-mail: J.Atack@Sussex.ac.uk

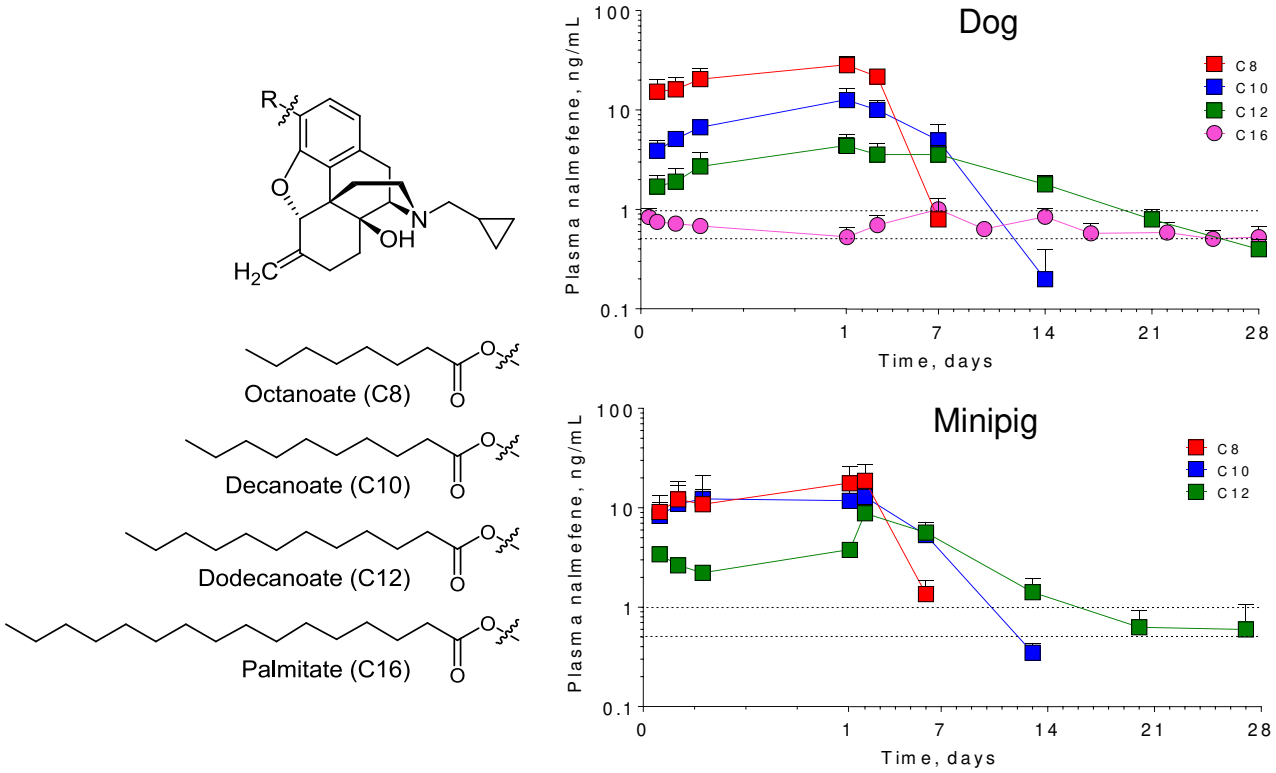
ABSTRACT

Nalmefene is an opioid antagonist which as a once-a-day tablet formulation has recently been approved for reducing ethanol intake in alcoholic subjects. In order to address the compliance issue in this patient population, a number of potential nalmefene prodrugs were synthesized with the aim of providing a formulation that could provide plasma drug concentrations in the region of 0.5-1.0 ng/mL for a one-month period when dosed intramuscular to dogs or minipigs. In an initial series of studies, three different lipophilic nalmefene derivatives were evaluated: the palmitate (C16), the octadecyl glutarate diester (C18-C5) and the decyl carbamate (CB10). They were administered intramuscularly to dogs in a sesame oil solution at a dose of 1 mg-eq. nalmefene/kg. The decyl carbamate was released relatively quickly from the oil depot and its carbamate bond was too stable to be used as a prodrug. The other two derivatives delivered a fairly constant level of 0.2-0.3 ng nalmefene/mL plasma for one month and since there was no significant difference between these two, the less complex palmitate monoester was chosen to demonstrate that dog plasma nalmefene concentrations were dose-dependent at 1, 5 and 20 mg-eq. nalmefene/kg. In a second set of experiments, the effect of the chain length of the fatty acid monoester promoieties was examined. The increasingly lipophilic octanoate (C8), decanoate (C10) and dodecanoate (C12) derivatives were evaluated in dogs and in minipigs, at a dose of 5 mg-eq. nalmefene/kg and plasma nalmefene concentrations were measured over a four-week period. The pharmacokinetic profiles were very similar in both species with C_{max} decreasing and T_{max} increasing with increasing fatty acid chain length and the target plasma concentrations (0.5-1.0 ng/ml over a month-long period) were achieved with the dodecanoate (C12) prodrug. These data therefore demonstrate that sustained plasma nalmefene concentrations can be achieved in both dog and minipig using nalmefene prodrugs and that the pharmacokinetic profile of nalmefene can be tuned by varying the length of the alkyl group.

Keywords: Nalmefene; opioid; extended-release; prodrug; pharmacokinetics

Chemical compounds studied in this article: Nalmefene (PubChem CID: 5284594)

GRAPHICAL ABSTRACT



Introduction

Alcohol-use disorders are reported to occur in 4-8% of the adult population in the US and have been estimated to be responsible for around 100,000 preventable deaths per year. The current FDA-approved pharmacological treatments for alcohol addiction include disulfiram (Antabuse®), acamprosate (Campral®), oral naltrexone (ReVia®) and extended-release naltrexone (Vivitrex®, now known as Vivitrol®) [1,2]. Disulfiram, which was first identified in 1939 based on an alcohol aversion observed in workers in the rubber industry in Ohio, prevents alcohol metabolism, resulting in an accumulation and causes alcohol aversion via a toxic response (flushing, hypotension, nausea and vomiting). The mechanism of acamprosate is poorly defined but is thought to be associated with an NMDA-related mechanism. Compared to acamprosate, the pharmacological actions of naltrexone are well understood and are related to its antagonism of opioid receptors. Recently, the European Medicines Agency (EMA)'s Committee for Medicinal Products for Human Use (CHMP) recommended a marketing authorization for nalmefene (17-*N*-cyclopropylmethyl-3,14-β-dihydroxy-4,5-α-epoxy-6-methylene-morphinan hydrochloride, also known as nalmetrene, NIH 10365 or 6-desoxy-6-methylene naltrexone; Tradename, Selincro®), which is structurally related to both naloxone and naltrexone (Figure 1A; [3]), which was granted in February 2013 [2,4] while the French Health agency has granted a "temporary recommendation for use" for the $GABA_B$ receptor agonist baclofen [5].

Nalmefene is an opioid receptor antagonist that binds to mu and kappa opioid receptors with an affinity (~0.3 nM) that is around 5-10 fold higher than naloxone and is well-tolerated in man following either i.v. or oral dosing [6,7] and has no abuse potential [8]. Although structurally-related to naltrexone, nalmefene is different in several important respects. First, although sharing a similar opioid receptor subtype *binding* selectivity with naltrexone, nalmefene possesses certain differences in subtype *efficacy* that may confer pharmacological advantages [9,10] that appear to translate into the clinic [11]. Hence, nalmefene is a partial agonist at kappa opioid receptors whereas naltrexone is an antagonist [12]. This results in nalmefene producing a greater suppression of ethanol intake relative to naltrexone [10]. Second, nalmefene is primarily eliminated by conjugation as the glucuronide [6,7] whereas naltrexone is metabolized to produce 6β-naltrexol [13,14], the latter of which is thought to be related to side-effects, particularly nausea [15], associated with naltrexone [16]. Third, the pharmacokinetic half-life in man is longer for nalmefene compared to naltrexone [17], which is an important consideration for the i.v. administration of opioid antagonists to block opioid-related effects [18] but is less of an issue for an extended-release formulation. Hence, nalmefene has a plasma half-life of around 8-11 h [6,7,19,20] that is appreciably longer than either

naloxone or naltrexone (half-lives in the region of 1 h and 3-10 h, respectively [17,21,22]). The longer half-life of nalmefene compared to naloxone is reflected in a longer duration of mu opioid receptor occupancy [23]. Fourth, nalmefene does not have the “black box” warning of hepatotoxicity that is found on the package insert of naltrexone and was responsible for 11 out of 614 subjects receiving 100 mg naltrexone per day having elevated plasma liver enzyme levels in the COMBINE study [24].

In alcohol-dependent subjects, compliance is a major issue [25] and is a key determinant in the relatively low rate of naltrexone prescriptions [26]. In disorders where compliance is an issue, extended-release or depot formulations of clinically proven drugs provides a valuable treatment option. A variety of approaches can be employed to provide a sustained exposure to drug [27,28], of which administration of the pharmacologically-relevant compound as a prodrug is but one. The aim of the current investigation was therefore to identify a formulation that could provide a one-month, sustained-release of nalmefene. Our approach was based on the successful strategy employed for antipsychotics, for example haloperidol decanoate [29,30] and paliperidone palmitate [31,32], in which a lipophilic prodrug in an oil depot formulation is administered intramuscularly [33,34]. Our target plasma concentrations of 0.5-1.0 ng/ml since were based upon the observation that a plasma nalmefene concentration of 0.34 ng/ml occupies 50% of human brain μ opioid receptors [35,36] and that 60-90% levels of occupancy required for efficacy [36].

Materials and Methods

Synthesis of prodrugs

The general scheme for the coupling of nalmefene with the appropriate acid chlorides, to obtain esters, or with isocyanate to obtain a carbamate (Fig. 1B) was used to produce a variety of different prodrugs (Fig. 1C). A more detailed analysis of the synthesis of the various prodrugs under evaluation is provided in the Supplemental Information.

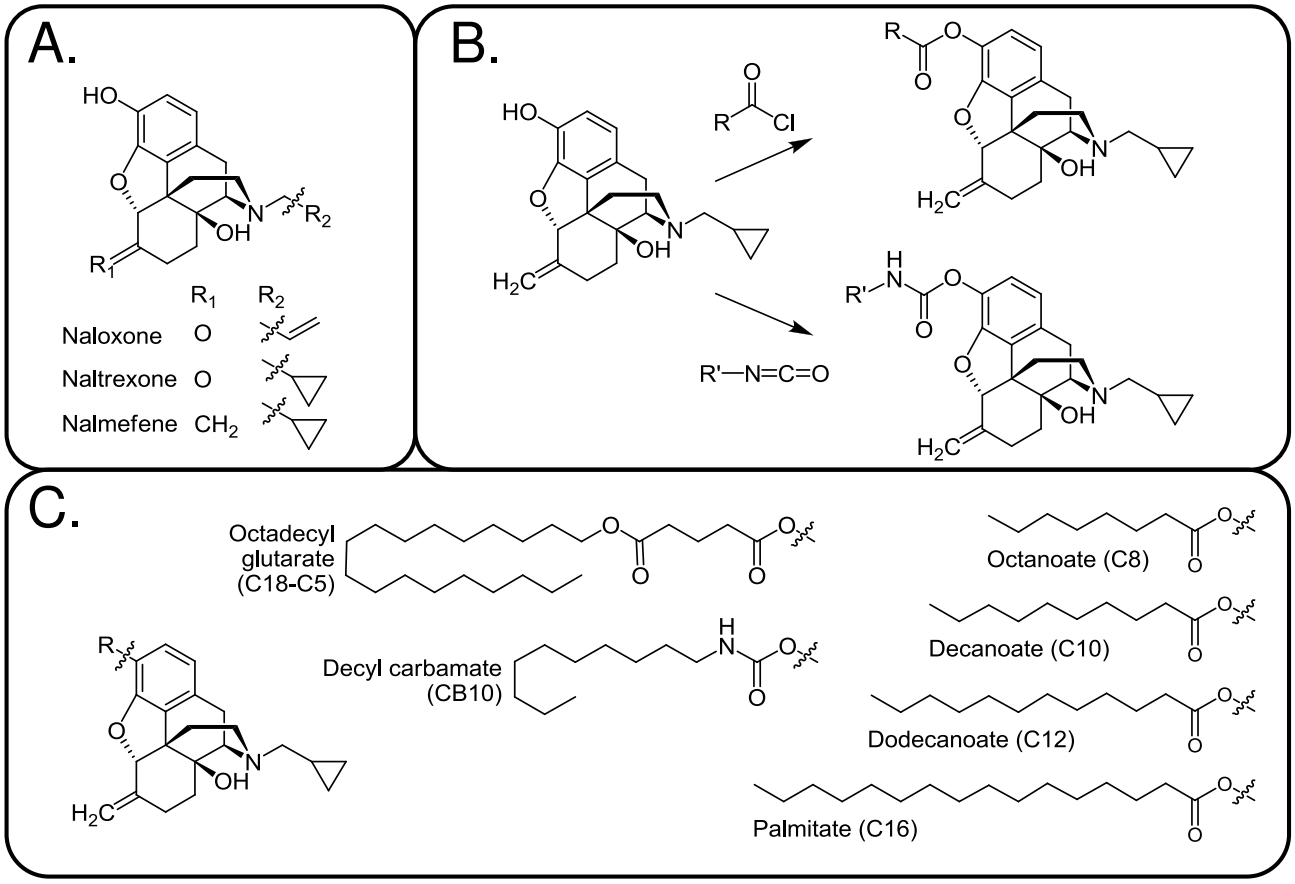


Fig. 1. Compound structures. A. Comparison of the structures of naloxone, naltrexone and nalmefene. Nalmefene is the 6-methylene analogue of naltrexone. B. General scheme summarizing the synthesis of nalmefene prodrugs. C. Comparison of the lipophilic side chains included in the various nalmefene prodrugs.

In Vivo Studies

All studies were approved by the local animal care committee and all studies were conducted in facilities accredited by national institutions adhering to AAALAC guidelines.

Intravenous (i.v.), oral (p.o.) and intramuscular (i.m.) kinetics of nalmefene in dog Three to six male CEDS beagle dogs (1 to 2 years old and body weight from 7 to 15 kg) were included per treatment group. They were fed an extruded maintenance diet (Sniff®), except on the day of dosing when they were food-restricted. Nalmefene was formulated as a saline solution (9 mg/mL NaCl) at concentrations of 1.5, 4 or 0.4 mg/mL for the i.v., oral and i.m. routes, respectively. Doses were 0.3 mg/kg (0.2 mL/kg) for the i.v. route, 2 mg/kg (0.5 mL/kg) for the oral route and 0.02 mg/kg (0.05 mL/kg) for the i.m. (m. biceps femoris) route. Blood samples were taken from the jugular vein at 5, 15 and 30 min and 1, 2, 4, 6 and 8 after i.v. dosing, at 30 min and 1, 2, 3, 4, 6 and 8 h after p.o. dosing and 1, 2 and 4 h after i.m. dosing.

Comparison of the palmitate (C16), octadecyl glutarate (C18-C5) and decyl carbamate (CB10) prodrugs of nalmefene Three male Harlan beagle dogs (5 to 8 years old and body weight from 7 to 16 kg) were included per treatment group and were fed an extruded maintenance diet (Sniff®). In the initial, 1 mg-eq./kg study, dogs were given 0.05 mL/kg i.m. (m. biceps femoris) of a 20 mg nalmefene equivalent/mL solution of either the palmitate (C16), octadecyl glutarate (C18-C5) and decyl carbamate (CB10) prodrug of nalmefene formulated in sesame oil. Blood samples were taken from the jugular vein at 1, 2, 4, 7 and 24 h after dosing on day 1 and at around 8 a.m. on days 2, 3, 6, 8, 10, 13 (or 15), 17, 20, 24 and 27. In a second study, dogs were given 5 mg-eq./kg i.m. of the palmitate (C16) prodrug of nalmefene, formulated as a solution in sesame oil, at a concentration of 12.5 mg-eq. nalmefene/mL. The dosing volume was 0.4 mL/kg, spread over two injection sites (left and right m. biceps femoris). Blood samples were taken from the jugular vein at 1, 2, 4, 7 and 24 h on day 1 and twice weekly thereafter until 11 weeks after dosing.
In the third study, 50 and 200 mg/mL nalmefene equivalent solutions of the palmitate nalmefene prodrugs were prepared in sesame oil and dosed intramuscularly at a volume of 0.1 mL/kg, at a single injection site (left m. biceps femoris). Blood samples were taken from the jugular vein at 1, 2, 4, 7 and 24 h and on a weekly base afterwards until 14 weeks post-dose. No injection site reactions were observed in any of the dogs.

Comparison of C8, C10 and C12 acyl chain lengths in dogs and minipigs Six female Harlan beagle dogs (5 to 8 years old and body weight from 10 to 21 kg) and four male Göttingen minipigs (1 to 2 years old and body weight from 22 to 30 kg) per treatment group were included. The prodrugs were formulated as solutions in sesame oil at a concentration of 50 mg-eq./mL and the formulations were dosed intramuscularly in the biceps femoris at a volume of 0.1 mL/kg (5 mg-eq./kg). Blood samples were taken from the jugular vein at 2, 4, 7 and 24 h after dosing and on a weekly basis until 4 weeks following dosing. No injection site reactions were observed in any of the dogs or minipigs.

Preparation of plasma samples and bioanalysis

Blood samples (2 mL on NaF and K-oxalate) were kept on ice for up to 30 min after sampling and then centrifuged for 10 min at 2000g at 4°C. Plasma was then removed and stored at -70°C until subsequent LC-MS/MS analysis. All plasma samples were analysed individually for nalmefene. The plasma samples from the dog studies were also analysed for the respective prodrugs that were administered.

Plasma aliquots were basified with 0.1 M borax after addition of internal standard solution (oxycodone or naltrexone). Next, the samples were extracted with either heptane/iso-amyl alcohol

95/5 (v/v) or with 1-chlorobutane. After extraction, the organic layer was evaporated to dryness under a nitrogen stream at 65°C. The residue was reconstituted in 50-100 µL acetonitrile, whereafter 100 µL 0.01 M ammonium acetate were added. The containers were centrifuged, and the content was transferred to an HPLC vial.

An aliquot was injected onto an LC-MS/MS system, using a 3 µm Polaris C18 column (50 x4.6 mm; Varian), which was eluted at 1 mL/min using an ammonium acetate 0.01 M /methanol gradient. Nalmefene and the internal standard eluted within this gradient. After the gradient, a step-gradient to ammonium acetate 0.01 M /methanol/THF 10/50/40 (v/v/v) was employed, and this mobile phase composition was maintained for 4 minutes in order to elute the pro-drugs from the column. For the detection, an API-4000 (AB Sciex) triple-quadrupole mass spectrometer was used in the positive ion electrospray mode in the MRM (multiple reaction monitoring) mode. For nalmefene, m/z 340.2 was chosen as mother ion and m/z 322.0 as daughter ion. The internal standard and prodrugs were also monitored in the MRM mode. The mother/daughter ions depended upon the internal standard that was chosen for the analysis and the prodrug that needed to be analyzed.

Data analysis

Individual plasma concentration-time profiles were subjected to a pharmacokinetic analysis using validated WinNonlin® software. A non-compartmental analysis using the lin/log trapezoidal rule with lin/log interpolation was used for all data.

Results

Pharmacokinetics of nalmefene in dogs following bolus dosing

Fig. 2 shows the plasma pharmacokinetic profiles of nalmefene following i.v. and oral dosing in dogs. The elimination half-life in dog was 0.85 and 1.5 h following i.v. and p.o., respectively with the former comparing to a value of 0.75 h in rat [37]. In man, i.v. half-life values of between 8 to 11 h were reported for doses of 1 or 2 mg [20] or 2-24 mg [6], although a shorter half-life (4.6 h) was observed after a low dose (30 µg i.v.; [38]). In dogs, the half-life of nalmefene after oral dosing, 1.5 h, was longer than that reported in rats (0.15 h; [39]) and both rat and dog half-lives were considerably shorter than in man with values of 7-15 h being reported after oral dosing of 50-300 mg [7,19] or 12.7 h derived from a population pharmacokinetic analysis of various i.v. and p.o. Phase 1 studies of nalmefene [36]. In dogs, the oral bioavailability of 1.3% is low and contrasts with the

value of 40% reported in man [7]. Given the limited number of data points following i.m. dosing, the elimination half-life estimated from these data, 0.92 h, should be viewed with caution. Nevertheless, these data do demonstrate that following dosing in an aqueous vehicle, there is no sustained release of nalmefene and they serve as a baseline for comparisons with i.m. injections of the various nalmefene prodrugs.

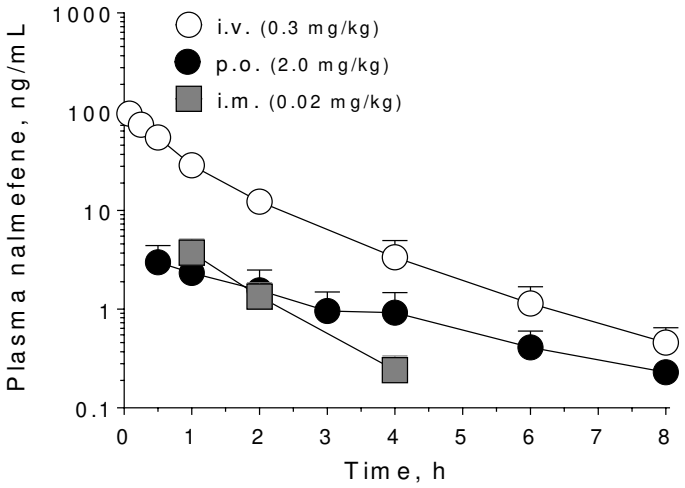


Fig. 2. Dog plasma pharmacokinetics of nalmefene following i.v., i.m. and p.o. dosing. Doses for the i.v., p.o. and i.m. routes were 0.3, 2 and 0.02 mg/kg, administered as aqueous solutions of 1.5, 4 and 0.4 mg/mL in respective dose volumes of 0.2 mL/kg, 0.5 mL/kg and 0.05 mL/kg. Values shown are mean + SD (n=3-6).

Comparison of different prodrug types

Initial studies focused on the plasma pharmacokinetics of nalmefene following i.m. injection (1 mg/kg nalmefene-equivalent) of the decyl carbamate (CB10), octadecyl glutarate (C18-C5) and palmitate (C16) ester prodrugs of nalmefene (Fig. 3A). These prodrugs were dissolved in sesame oil by analogy with the formulation of haloperidol decanoate [40,41] at a concentration of 20 mg-eq. nalmefene/ml. The pharmacokinetic profiles show that on Day 1, both the octadecyl glutarate (C18-C5) and the decyl carbamate (CB10) prodrug showed some “burst effect” in the first few hours of the experiment (Fig. 3A), which is probably due to some unconjugated nalmefene which is still present in the corresponding prodrug (C_{max} values of 4.0 and 1.5 ng/mL, respectively, at 1 h). In contrast, the nalmefene concentrations produced from the palmitate prodrug were relatively constant (C_{max} <0.2 ng/mL), with the caveat that plasma samples from the palmitate prodrug-treated animals were available only for 1 and 24 h after dosing. After Day 1, the diester and palmitate prodrugs maintained relatively constant nalmefene plasma concentrations (~0.2-0.3 ng/mL) but, in contrast, the concentrations of nalmefene originating from the carbamate prodrug declined continuously over the 4-wk period and fell below the level of quantification (0.1 ng/mL) 24 days after measurement.

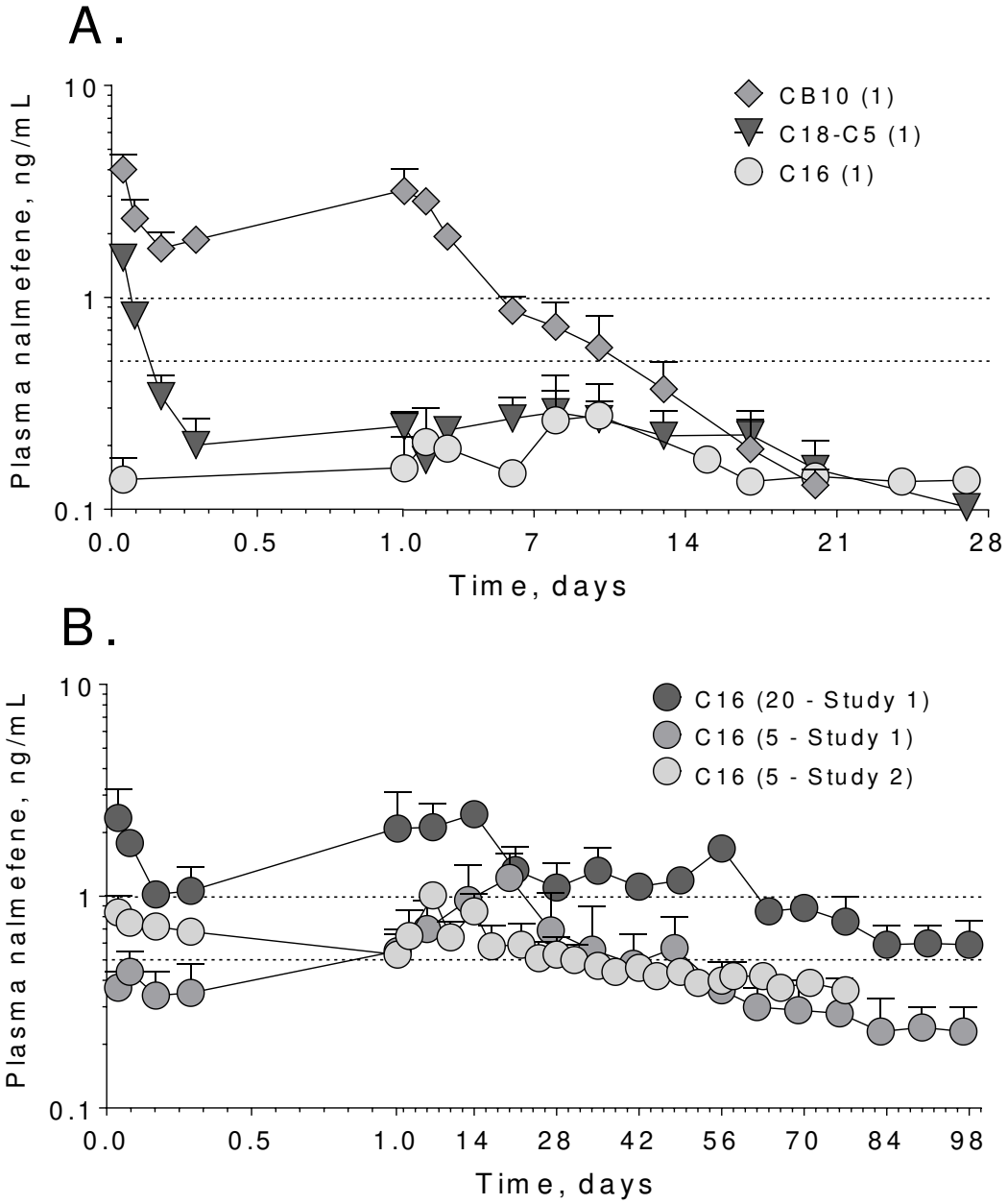


Fig. 3. Dog plasma nalmefene concentration versus time profiles after intramuscular administration of: A. 1 mg-eq. nalmefene/kg of decyl carbamate (CB10), octadecyl glutarate (C18-C5) and palmitate (C16) nalmefene prodrugs. B. 5 and 20 mg-eq. nalmefene/kg of the palmitate prodrug with the 5 mg-equivalent dose being administered on two separate occasions (palmitate 5-I and 5-II). The dashed lines represent the target plasma concentrations of 0.5-1.0 ng/mL estimated to be required for efficacy in man. Values shown are mean + SD (n=3/group).

In a subsequent study (Fig. 3B), the kinetics of the nalmefene release from 5 and 20 mg-eq. nalmefene/kg in the palmitate prodrug were evaluated over a 98-day period (Study 1). In this study, plasma nalmefene concentrations were roughly dose-dependent with, for example, Day 1 levels being 0.4-0.5 and 1-2 ng/mL for the 5 and 20 mg-eq. nalmefene/kg doses, respectively. Importantly, after

Day 1, the 5 mg-eq. nalmefene/kg produced plasma concentrations within the 0.5-1.0 ng/mL nalmefene target range. A replication study was performed over a 77-day period using the 5 mg-eq. nalmefene/kg dose to assess the reproducibility of these data (Study 2). In this study, the Day 1 plasma concentrations (0.5-0.8 ng/mL) were slightly higher than the initial study (0.4-0.5 ng/mL) but thereafter, and as with Study 1, plasma nalmefene concentrations remained above the 0.5 ng/mL target for the initial 28-day period.

Collectively, these data clearly demonstrated that by synthesizing a lipophilic prodrug of nalmefene, it was possible to achieve a sustained exposure in dogs. From these data, it was concluded that neither the decyl carbamate nor the diester octadecyl glutarate prodrugs conferred an advantage over the palmitate prodrug and therefore further efforts were focused upon monoester prodrugs with the aim of determining the effect of the prodrug alkyl chain length on plasma nalmefene concentrations. In addition, pharmacokinetic experiments were extended from dogs to include a second species, namely the minipig.

Effect of nalmefene alkyl chain length on dog and mini-pig plasma nalmefene concentrations

To evaluate the effect of the length of the fatty acid on the pharmacokinetic profile three monoesters of nalmefene were synthesised in addition to the palmitate (C16): the octanoate (C8), the decanoate (C10) and the dodecanoate (C12). The concentration of the prodrugs dissolved in the sesame oil formulation was increased to 50 mg-eq. nalmefene/mL to give a dose of 5 mg-eq./kg in order to achieve steady-state concentrations above the target therapeutic concentration of 0.5 ng/mL. These prodrugs were evaluated in dogs (Fig. 4A) and minipigs (Fig. 4B). The hypothesis was that the alkyl group chain length would determine the rate of release from the sesame oil vehicle with prodrugs with longer alkyl chain lengths having increased lipophilicity, and therefore a lower rate of release from the sesame oil carrier, relative to those with shorter chain lengths. This indeed proved to be the case with the plasma levels of nalmefene being much higher during the first 24 h for the C8 compared to the C12 prodrug, not only in dogs (Fig. 4A) but also minipigs (Fig. 4B).

The nalmefene prodrug alkyl group chain length not only had a large effect on the plasma nalmefene concentrations on Day 1 but also markedly influenced the plasma nalmefene concentrations thereafter. For example, in dog plasma the nalmefene concentrations after dosing with the C8 prodrug fell from 28.5 ± 6.5 ng/mL at the end of Day 1 to only 0.8 ± 0.5 ng/mL on Day 7 whereas concentrations following dosing with the C12 prodrug were relatively stable between Days 1 and 7 (respective plasma nalmefene concentrations of 4.4 ± 1.3 and 1.8 ± 0.4 ng/mL).

A comparison of the plasma nalmefene concentrations in dogs and minipigs showed that the plasma nalmefene kinetic profiles of the different prodrugs were not only qualitatively similar across species but also that the absolute values were comparable. This suggests that the main determinant of plasma nalmefene concentrations, namely release from the sesame oil vehicle, is similar in dogs and minipigs. As for the prodrug itself, in the dog study, the plasma concentration for the unhydrolysed octanoate (C8), the decanoate (C10) and the dodecanoate (C12) prodrugs were also measured. The first two stayed below the lower level of quantification (0.5 ng/mL). The dodecanoate derivative was detected and its concentration was in general 20% of that of nalmefene itself at 7h after dosing and it decreased to less than 10% of the nalmefene levels from 24h after dosing onwards.

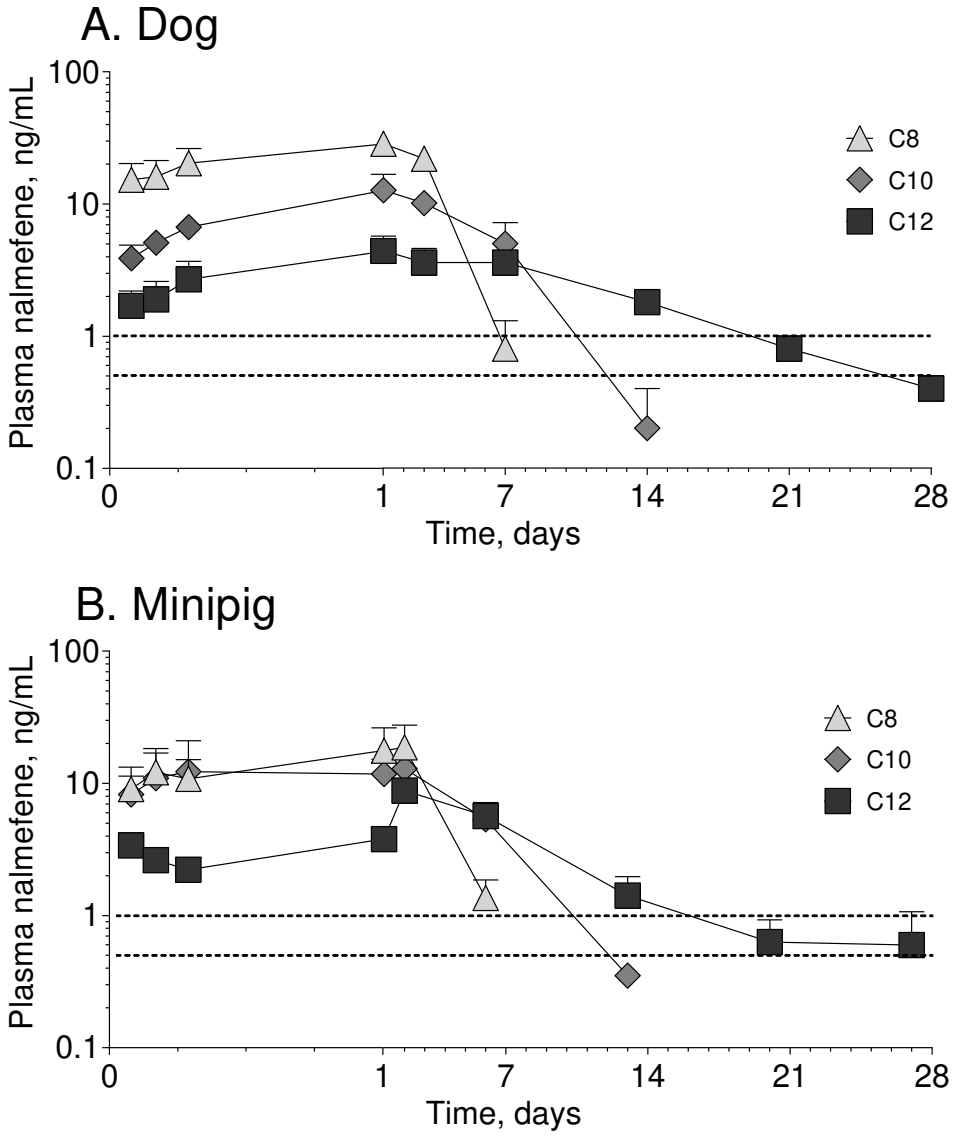


Fig. 4. Effect of alkyl chain length upon plasma nalmefene concentrations. A. Dog and B. Mini-pig plasma nalmefene concentrations following i.m. dosing of 0.1 mL/kg of a 50 mg-eq. nalmefene/kg solution (in a sesame oil vehicle) of each prodrug into different cohorts of animals. The dashed lines represent the target plasma concentrations of 0.5-1.0 ng/mL estimated to be required for efficacy in man. Values shown are mean + SD (n=6/group for dog, n=4-6/group for mini-pig).

Discussion

Clinical uses of nalmefene

The i.v. formulation of nalmefene (trade-name of Revex®) has efficacy in treating opioid overdose [42] and antagonizes opioid-related effects with a longer duration than naloxone [18]. Consequently i.v. nalmefene has utility in the emergency room [17], in which setting it offers an advantage over i.v. naloxone (trade name Narcan®) in that it reduces the possibility of the re-emergence of opioid-related effects caused by clearance of the antagonist [17].

Oral nalmefene was initially described to have efficacy in alcoholic subjects by improving the time to relapse to heavy drinking [43], reducing the craving [44], the number of heavy drinking days [45]. These data have recently been extended to show in the ESENSE1, ESENSE2 and SENSE trials that oral nalmefene produces a reduction in alcohol consumption [46-49] and resulted in nalmefene being licensed by the EMA for "the reduction of alcohol consumption in adult patients with alcohol dependence who have a high drinking risk level without physical withdrawal symptoms and who do not require immediate detoxification". Additional *post-hoc* studies on these data have demonstrated that nalmefene is well-tolerated with no major safety issues [50] and is associated with an improvement in health-related quality of life indices [51] and a reduction in mortality risk [52]. Finally, nalmefene in conjunction with psychosocial support is cost effective relative to psychosocial support alone [53,54].

Compliance is a significant issue in alcohol-dependent subjects [25,26] and it is not unreasonable to assume that given the nature of the patient population, compliance may also be an issue for oral nalmefene in the treatment of alcoholism since it is prescribed for use on an as-needed basis. Accordingly, it is desirable to develop a depot formulation of nalmefene that would reduce the potential risk of poor compliance.

Selecting a target plasma concentration of nalmefene

Nalmefene is a potent opioid antagonist with a single 2-mg i.v. dose being able to completely block and maintain a fentanyl (2 μg/kg i.v.)-induced respiratory depression for a full 8-h period [19], after which time plasma nalmefene concentrations are in the region of 0.7-1.0 ng/mL [6,20]. The potency of nalmefene is further reflected in the measurement of mu opioid receptor occupancy as measured using [^{11}C]carfentanil PET [23,35]. Hence, after a single 20-mg oral dose of nalmefene, occupancy was around 85% and 50% at 26 and 50 h after dosing, respectively [35], corresponding to plasma concentrations in the region of 2 and 0.8 ng/mL [7,35]. Furthermore, using [^{11}C]carfentanil and a

dual-detector system, a 1-mg i.v. dose of nalmefene gave respective occupancy values of 96% and 86%, 4 and 8 h after dosing [23] at which times the corresponding plasma nalmefene concentrations would be predicted to be in the region of ~1 and ~0.4 ng/mL [6,20]. Consequently, a plasma nalmefene concentration of 0.5-1 ng/mL was chosen as a target for an extended-release formulation since it would be expected to give sustained mu opioid receptor occupancy in the region of >85-90%.

Development of long-acting nalmefene

In order to develop an extended release formulation of nalmefene, a prodrug approach was chosen since it has been successful in the development of extended release antipsychotic medications, such as haloperidol decanoate [33]. The general strategy is to identify a lipophilic derivative of nalmefene that dissolves in an oil from which it will diffuse out steadily over a period of one month, presumably in a process involving the lymphatic system [34]. In this regard, the phenol functionality of nalmefene is ideal as a synthetic group on which to attach lipophilic promoieties. Once it has left the formulation (which is administered intramuscularly), the prodrug has to be cleaved into nalmefene and the non-toxic prodrug moiety. As with haloperidol decanoate model, sesame oil was chosen as solvent for the depot formulation of these prodrugs as it is regarded as safe and well tolerated for intramuscular and subcutaneous administration. It is accepted by the regulatory bodies as an excipient and it is as such used for a number of drugs that are on the market like estradiol valerate, hydroxyprogesterone caproate, testosterone enanthate, nandrolone decanoate and the decanoate or enanthate esters of fluphenazine.

In an initial study, three different prodrugs were prepared that varied with respect to their chain lengths and the functional group that linked them with the alcohol of the drug. Of these prodrugs, the carbamate bond proved to be too stable – and therefore unsuitable – since the carbamate prodrug itself was the only prodrug that was found in quantifiable levels in plasma. The diester linkage within the octadecyl glutarate (C18-C5) conferred no advantage relative to the monoester of the palmitate (C16) and accordingly the monoester prodrugs were chosen for further evaluation. Additional prodrugs with a single ester linker were synthesized and administered in sesame oil to evaluate the effect of alkyl group chain length upon plasma pharmacokinetics in dogs and minipigs. Both species had comparable plasma nalmefene pharmacokinetic profiles for each of the different prodrugs with a clear trend being observed in which C_{max} diminished and T_{max} increased by elongating the fatty acid promoiety with the dodecanoate (C12) prodrug having a “flatter” and more sustained plasma nalmefene pharmacokinetic profile than either the octanoate (C8) or decanoate (C10) prodrugs (Figure 4). An important feature of the plasma nalmefene pharmacokinetic profiles

produced by these various prodrugs is the relative lack of inter-individual variability. For example, the majority of the coefficients of variation are ≤50%, with most being ≤25%, indicating that in this admittedly relatively homogeneous population of dogs and minipigs, plasma nalmefene exposures are similar between animals.

Both the dodecanoate and the palmitate meet the study criterion of being able to produce a sustained therapeutic level for 4 weeks at a dose of 5 mg-eq. nalmefene/kg. For a one-month depot formulation of nalmefene the dodecanoate is preferred, as the depot is almost completely depleted after 1 month, while the palmitate still releases significant amounts of nalmefene for at least 2.5 months. For this reason, it is more likely that dose escalation will occur after multiple dosing with the palmitate prodrug. Once-monthly dosing is seen as optimal, as it allows regular follow-up by a physician. By not making the period of sustained delivery of an opioid antagonist too long, scheduling possible elective surgery, in which opiate analgesics are required, becomes more practical [55].

Additional extended-release formulations of nalmefene have been described based on poly-(lactide-co-glycolide) microspheres [39,56] or nalmefene blended into ethylene vinyl acetate rods [57]. However, for the former, plasma drug concentrations were quite variable (e.g., concentrations of nalmefene at Days 3, 5 and 21 were 111, 35 and 69 ng/ml, respectively), and for the latter there was a marked, approximately 10-fold decrease in plasma nalmefene concentrations over the initial 4-wk period after implanting in rats [57]. In contrast, the single ester prodrugs described in the present study, especially the palmitate prodrug, produce much more constant plasma nalmefene concentrations and it was possible modify the pharmacokinetic profile by altering the length of the lipid side chain.

In summary, these data show that alkyl ester prodrugs of nalmefene can produce sustained plasma concentrations of nalmefene in the region of 0.5-1.0 ng/mL for several weeks in dogs and mini-pigs, with the kinetics being dictated by the length of the alkyl group. Moreover, a dose of 5 mg/kg equivalent of the dodecanoate single-ester prodrug produces plasma nalmefene concentrations (0.5-1.0 ng/ml) sufficient to achieve the 60-90% levels of μ opioid receptor occupancy required for clinical efficacy [36] in alcohol dependence [4] and potentially other disorders [58,59].

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SUPPLEMENTAL INFORMATION

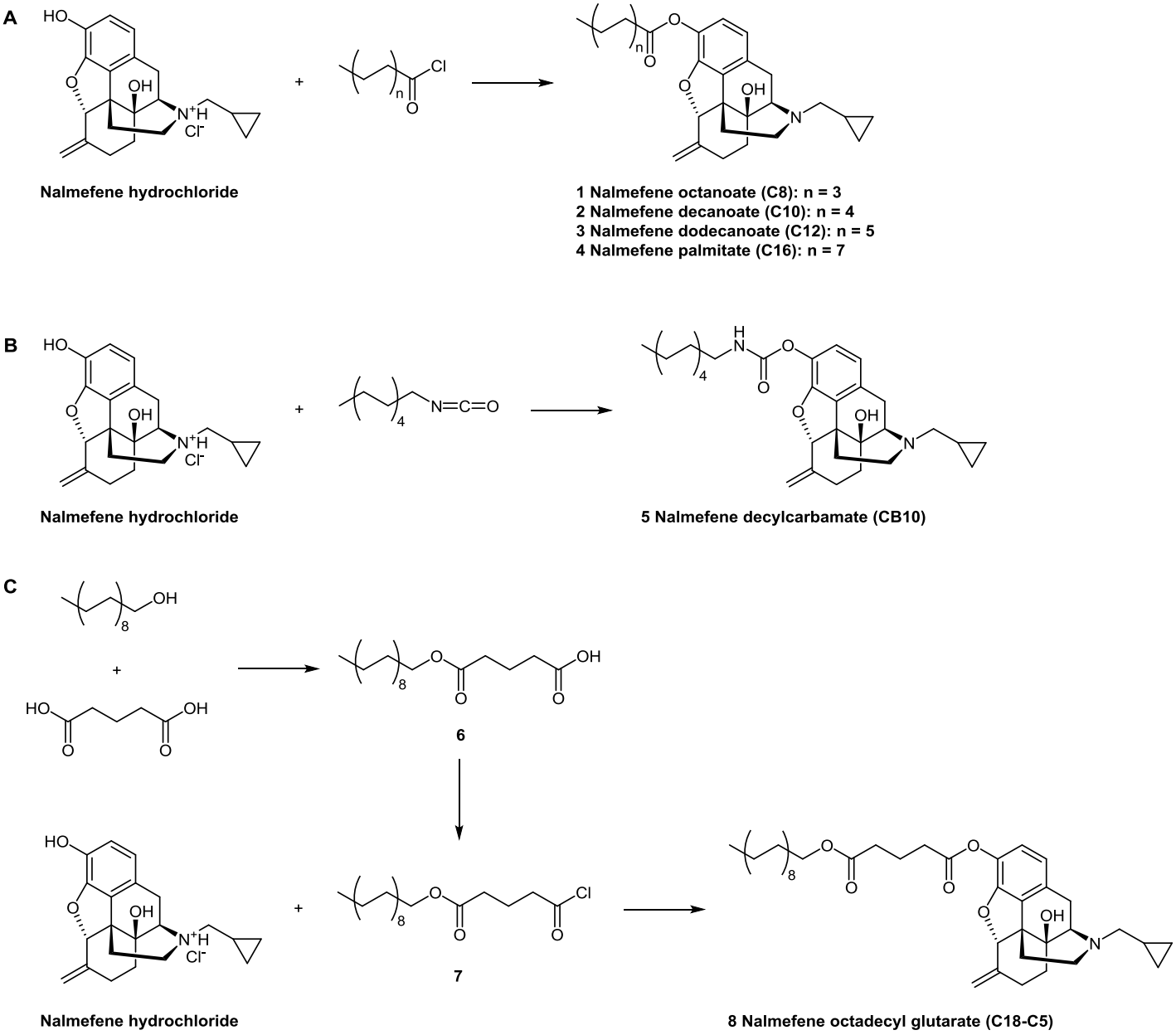

Figure S1. 2. Synthetic schemes of the different nalmefene prodrugs. A, the fatty acid ester prodrugs of nalmefene with different chain lengths: nalmefene octanoate (C8; n = 3), nalmefene decanoate (C10; n = 4), nalmefene dodecanoate (C12; n = 5) and nalmefene palmitate (C16; n = 7). B, the nalmefene decylcarbamate (CB10) prodrug. C, the nalmefene octadecyl glutarate (C18-C5) prodrug.

Chemicals. Nalmefene hydrochloride was purchased from Diosynth with a purity of well above 98%. All other reagents were obtained from Acros or Aldrich with a purity of 98% or more.

Synthesis and Characterization of the Fatty Acid Ester Nalmefene Prodrugs.

Nalmefene octanoate (C8). Octanoyl chloride (2.27 ml, 13.3 mmol) was added dropwise over 30 minutes at ambient temperature to a stirred mixture of nalmefene hydrochloride (5.00 g, 13.3 mmol),

toluene (100 ml) and triethylamine (4.08 ml, 29.3 mmol) under an inert atmosphere. Stirring at ambient temperature was continued for 16 hours. Octanoyl chloride (0.15 ml, 0.9 mmol) was added extra. The solution was stirred for 4 additional hours at ambient temperature to complete the conversion. Afterwards, the reaction mixture was washed with water (400 ml). After phase separation, the organic layer was dried with magnesium sulfate. Concentration of the filtrate under reduced pressure afforded nalmefene octanoate (**1**) (5.98g, 96%); oil at ambient temperature; ^{1}H NMR 400 MHz [$(CD_3)_2SO$] δ 0.02 to 0.18 (m, 2H), 0.38 to 0.55 (m, 2H), 0.76 to 0.94 (m, 4H), 1.18 to 1.41 (m, 10H), 1.53 (dt, J = 12.78, 3.30 Hz, 1H), 1.58 to 1.70 (m, 2H), 1.96 (td, J = 12.09, 3.78 Hz, 1H), 2.07 (dt, J = 13.72, 3.15, 3.02 Hz, 1H), 2.24 (td, J = 12.59, 5.04 Hz, 1H), 2.31 to 2.40 (m, 2H), 2.45 to 2.50 (m, 1H), 2.54 (t, 2H), 2.53 to 2.61 (m, 1H), 2.65 (dd, J = 11.83, 4.28 Hz, 1H), 3.03 (dd, J = 12.09, 6.55 Hz, 2H), 4.80 (d, J = 1.76 Hz, 1H), 5.03 (br s, 1H), 4.96 (s, 1H), 5.05 (d, J = 1.26 Hz, 1H), 6.66 (d, J = 8.31 Hz, 1H), 6.76 (d, J = 8.06 Hz, 1H). HRMS (electrospray - positive) calculated for $C_{29}H_{39}NO_4$ (MH^+) m/z 466.2957, found 466.2967. Analysis of the final product showed that nalmefene octanoate contained 0.21% of unconjugated nalmefene.

Nalmefene decanoate (C10). The same procedure as described for nalmefene octanoate was followed with decanoyl chloride. In this case no additional acid chloride was required to obtain a complete conversion. This afforded nalmefene decanoate (**2**) (6.31 g, 96%); oil at ambient temperature; ^{1}H NMR 400 MHz [$(CD_3)_2SO$] δ 0.09 to 0.15 (m, 2H), 0.43 to 0.52 (m, 2H), 0.82 to 0.89 (m, 4H), 1.15 to 1.42 (m, 14H), 1.53 (dt, J = 12.84, 3.40 Hz, 1H), 1.63 (quin, J = 7.30 Hz, 2H), 1.96 (td, J = 12.09, 3.78 Hz, 1H), 2.07 (dt, J = 13.60, 3.27 Hz, 1H), 2.24 (td, J = 12.53, 5.16 Hz, 1H), 2.30 to 2.40 (m, 2H), 2.44 to 2.49 (m, 1H), 2.54 (t, J = 7.55 Hz, 2H), 2.53 to 2.61 (m, 1H), 2.65 (dd, J = 11.83, 4.28 Hz, 1H), 3.03 (dd, J = 12.09, 6.55 Hz, 2H), 4.96 (br s, 1H), 4.80 (d, J = 1.76 Hz, 1H), 4.97 (s, 1H), 5.05 (d, J = 1.51 Hz, 1H), 6.66 (d, J = 8.06 Hz, 1H), 6.75 to 6.78 (m, 1H). HRMS (electrospray - positive) calculated for $C_{31}H_{43}NO_4$ (MH^+) m/z 494.3270, found 494.3265. Analysis of the final product showed that nalmefene decanoate contained 0.16% of unconjugated nalmefene.

Nalmefene dodecanoate (C12). The same procedure as described for nalmefene octanoate was followed with dodecanoyl chloride. In this case no additional acid chloride was required to obtain a complete conversion. This afforded nalmefene dodecanoate (**3**) (6.36 g, 92%); viscous oil at ambient temperature; ^{1}H NMR 400 MHz [$(CD_3)_2SO$] δ 0.08 to 0.16 (m, 2H), 0.44 to 0.51 (m, 2H), 0.81 to 0.89 (m, 4H), 1.15 to 1.42 (m, 18H), 1.53 (dt, J = 12.78, 3.30 Hz, 1H), 1.63 (quin, J = 7.43 Hz, 2H), 1.96 (td, J = 12.09, 3.53 Hz, 1H), 2.06 (dt, J = 13.60, 3.15 Hz, 1H), 2.24 (td, J = 12.46, 5.04 Hz, 1H), 2.29 to 2.40 (m, 2H), 2.45 to 2.49 (m, 1H), 2.53 (t, J = 7.68 Hz, 2H), 2.53 to 2.60 (m, 1H), 2.65 (dd, J = 11.96, 4.41 Hz, 1H), 3.03 (dd, J = 11.96, 6.67 Hz, 2H), 4.99 (br s, 1H), 4.80 (d, J = 1.76 Hz, 1H), 4.96 (s, 1H), 5.05 (d, 1H), 6.66 (d, J = 8.06 Hz, 1H), 6.76 (d, J = 8.06 Hz, 1H). HRMS (electrospray - positive) calculated for $C_{33}H_{47}NO_4$ (MH^+) m/z 522.3583, found 522.3590. Analysis of the final product showed that nalmefene decyl carbamate contained 0.37% of unconjugated nalmefene.

Nalmefene palmitate (C16). The same procedure as described for nalmefene octanoate was followed with palmitoyl chloride. In this case no additional acid chloride was required to obtain a complete conversion. This afforded nalmefene palmitate (**4**) (7.69 g, 95%); mp 44°C; ^{1}H NMR 400 MHz [(CD3)2SO] δ 0.08 to 0.16 (m, 2H), 0.43 to 0.53 (m, 2H), 0.81 to 0.89 (m, 4H), 1.14 to 1.42 (m, 26H), 1.53 (dt, J = 12.78, 3.65, 3.46 Hz, 1H), 1.59 to 1.67 (m, J = 7.55, 7.55, 7.55, 7.55 Hz, 2H), 1.96 (td, J = 12.02, 3.65 Hz, 1H), 2.07 (dt, J = 13.66, 3.08, 2.90 Hz, 1H), 2.24 (td, J = 12.53, 4.91 Hz, 1H), 2.35 (t, J = 6.04 Hz, 2H), 2.43 to 2.49 (m, 1H), 2.54 (t, J = 7.43 Hz, 2H), 2.53 to 2.61 (m, 1H), 2.62 to 2.68 (m, 1H), 3.03 (dd, J = 12.09, 6.55 Hz, 2H), 4.80 (d, J = 1.76 Hz, 1H), 4.91 (br s, 1H), 4.96 (s, 1H), 5.05 (d, J = 1.26 Hz, 1H), 6.66 (d, J = 8.06 Hz, 1H), 6.77 (d, J = 8.31 Hz, 1H). HRMS (electrospray - positive) calculated for $C_{37}H_{56}NO_4$ (MH^+) m/z 578.4209, found 578.4199. Elemental analysis calculated for $C_{37}H_{55}NO_4$: C, 76.91; H, 9.59; N, 2.42; found: C, 77.01; H, 9.96; N,1.89. Analysis of the final product showed that nalmefene palmitate contained 0.06% of unconjugated nalmefene.

Synthesis and Characterization of Nalmefene Decylcarbamate (CB10).

A suspension of nalmefene hydrochloride (20 g, 53.2 mmol), toluene (400 ml), triethylamine (8.9 ml, 63.8 mmol) and decyl isocyanate (13.3 ml, 63.8 mmol) was refluxed for 16 hours. 3 additional portions of decyl isocyanate were added (respectively 2.2 ml, 10.6 mmol; 2.2 ml, 10.6 mmol and 4.4 ml, 21.3 mmol) during the next 24 hours of reflux. After the reaction mixture was cooled to ambient temperature, it was washed with water (400 ml). The organic layer was dried with magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by

chromatography on silica gel (60 Å 25-40 μg); and elution with EtOAc, afforded nalmefene decylcarbamate (**5**) (17.4 g, 63%); oil at ambient temperature; ^{1}H NMR 400 MHz [$(CD_3)_2SO$] δ 0.06 to 0.17 (m, 2H), 0.41 to 0.53 (m, 2H), 0.78 to 0.91 (m, 4H), 1.22 to 1.31 (m, 16H), 1.40 to 1.48 (m, 2H), 1.52 (dt, J = 12.78, 3.56 Hz, 1H), 1.97 (td, J = 11.96, 3.53 Hz, 1H), 2.06 (dt, J = 13.60, 3.53 Hz, 1H), 2.23 (td, J = 12.46, 5.04 Hz, 1H), 2.35 (dd, J = 6.29, 3.78 Hz, 2H), 2.43 to 2.49 (m, 1H), 2.56 (dd, J = 18.88, 5.79 Hz, 1H), 2.65 (dd, J = 11.71, 4.15 Hz, 1H), 2.97 to 3.08 (m, 4H), 4.79 (d, J = 1.51 Hz, 1H), 4.90 (s, 1H), 4.94 (s, 1H), 5.10 (d, J = 1.01 Hz, 1H), 6.62 (d, J = 8.06 Hz, 1H), 6.75 (d, J = 8.31 Hz, 1H), 7.63 (t, J = 5.67 Hz, 1H). HRMS (electrospray - positive) calculated for $C_{32}H_{47}N_2O_4$ (MH^+) m/z 523.3536, found 523.3517. Elemental analysis calculated for $C_{32}H_{46}N_2O_4$: C, 73.53; H, 8.87; N, 5.36; found: C, 74.73; H, 9.45; N, 5.58. Analysis of the final product showed that nalmefene decyl carbamate contained 1.33% of unconjugated nalmefene.

Synthesis and Characterization of Nalmefene Octadecyl Glutarate.

Intermediate 6. A solution of 1-octadecanol (16.4 g, 60.6 mmol), toluene (800 ml), glutaric acid (80.1 g, 606 mmol) and p-toluenesulfonic acid (1.0 g, 6.1 mmol) was heated to 100°C for 16 hours. The reaction mixture was cooled to ambient temperature and washed with water. The organic layer was dried with magnesium sulfate, filtered and concentration to dryness afforded 5-(octadecyloxy)-5-oxopentanoic acid (**6**) (21.5 g, 92%); ^{1}H NMR 400 MHz [$CDCl_3$] δ 0.90 (t, J = 6.55 Hz, 3H), 1.19 to 1.41 (m, 30H), 1.59 to 1.68 (m, J = 7.05, 7.05, 7.05, 7.05 Hz, 2H), 1.93 to 2.03 (m, J = 7.05, 7.05, 7.05, 7.05 Hz, 2H), 2.41 (t, J = 7.30 Hz, 2H), 2.45 (t, J = 7.30 Hz, 2H), 4.09 (t, J = 6.67 Hz, 2H), 9.57 (br s, 1H).

Intermediate 7. Thionyl chloride (8.8 ml, 121.2 mmol) was added dropwise to a solution of 5-(octadecyloxy)-5-oxopetanoic acid (23.3 g, 60.6 mmol), toluene (233 ml) and triethylamine (8.5 ml, 60.6 mmol) at ambient temperature under an inert atmosphere. The mixture was heated for 2 hours at 80°C. After cooling to ambient temperature, the salts were filtered and washed with toluene. Concentration of the filtrate under reduced pressure afforded octadecyl 5-chloro-5-oxopentanoate (**7**) (24.4 g, 100%). The residue was used as such in the next step.

Nalmefene octadecyl glutarate (C18-C5). A solution of octadecyl 5-chloro-5-oxopentanoate (21.4 g, 53.2 mmol) in toluene (200 ml) was added dropwise over 1.5 hours to a suspension of nalmefene hydrochloride (20 g, 53.2 mmol), toluene (200 ml) and triethylamine (16.3 ml, 117.0 mmol). The reaction mixture was stirred at ambient temperature for 16 hours. It was washed with water (400 ml) and the aqueous layer was extracted twice with toluene before being discarded. The combined

organic layers were dried with magnesium sulfate and evaporated. The residue was triturated with methanol (100 ml). The precipitate was filtered and washed with methanol (100 ml). Drying for 16 hours at 50°C under reduced pressure afforded nalmefene octadecyl glutarate (**8**) (30.5 g, 81%); mp 47°C; ^{1}H NMR 400 MHz [$(CD_3)_2SO$] δ 0.06 to 0.17 (m, 2H), 0.42 to 0.54 (m, 2H), 0.80 to 0.89 (m, 4H), 1.14 to 1.34 (m, 32H), 1.49 to 1.60 (m, 3H), 1.83 to 1.92 (m, J = 7.30, 7.30, 7.30, 7.30 Hz, 2H), 1.97 (td, J = 11.90, 3.90 Hz, 1H), 2.07 (dt, J = 13.53, 3.34, 3.02 Hz, 1H), 2.24 (td, J = 12.59, 5.04 Hz, 1H), 2.35 (t, J = 6.04 Hz, 2H), 2.44 (t, J = 7.30 Hz, 2H), 2.47 to 2.53 (m, 1H), 2.54 to 2.60 (m, 1H), 2.60 (t, J = 7.18 Hz, 2H), 2.63 to 2.68 (m, 1H), 3.03 (dd, J = 11.96, 6.67 Hz, 2H), 4.02 (t, J = 6.55 Hz, 2H), 4.80 (d, J = 1.26 Hz, 1H), 4.90 (s, 1H), 4.97 (s, 1H), 5.05 (d, J = 1.01 Hz, 1H), 6.67 (d, J = 8.31 Hz, 1H), 6.78 (d, J = 8.31 Hz, 1H). HRMS (electrospray - positive) calculated for $C_{44}H_{68}NO_6$ (MH+) m/z 706.5047, found 706.5034. Elemental analysis calculated for $C_{44}H_{67}NO_6$: C, 74.85; H, 9.57; N, 1.98; found: C, 75.88; H, 10.13; N, 1.50. Analysis of the final product showed that nalmefene octadecyl glutarate contained 0.63% of unconjugated nalmefene.